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BIOCHEMICAL COMPOSITION AND NUTRITIONAL EVALUATION OF BARLEY RIHANE (HORDEUM VULGARE L.)

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#### **Abstract**

**Background**: Many experimental studies have suggested an important role for barley Rihane(BR)in the prevention of colon cancer and cardiovascular diseases. The objective of this study was to evaluate the physico-chemical properties and nutritional characterizations of BR compared to other varieties grown in Tunisia (Manel, Roho and Tej).

Material and Methods: Total, insoluble and soluble dietary fiber( $\beta$ -glucan),total protein, ash and some minerals of BR and Tunisian barley varieties were determined.

Results: The results revealed that BR is good source of dietary fiber mainly  $\beta$ -glucan compared to the other varieties. This variety is a relatively rich source of phosphorous and potassium and it contains many important unsaturated fatty acids. BR has higher nutritional value than other varieties.

Conclusion: Barley Rihane has significant nutritional characterizations compared to others Tunisian barleys varieties.

**Key words**: Barley Rihane; biochemical composition; nutritional properties; β-glucan; bioactive compounds; functional food.

**Abbreviations**: BR, Barley Rihane; LDL, low density lipoprotein; HDL, high density lipoprotein; AOM, azoxymethane; TBV, Tunisian barley varieties; TGW, thousand grain weight; SW, weight specific; TDF, total dietary fiber; IDF, insoluble dietary fiber; SDF, soluble dietary fiber; DM, Dry Matter.

#### Introduction

The past decade has witnessed intense interest in nutraceuticals or functional foods (Hosseinzadeh et al., 2015). Epidemiological studies have consistently shown that diets rich in whole grains are associated with a decreased risk of a number of chronic diseases such as coronary heart disease, type II diabetes and certain cancers (Susan et al. 2013). The beneficial effect of whole grains and cereal products has often been attributed to their functional or bioactifscomponents content. Foods rich in dietary fiber tend to be a rich source of many other health promoting components such as, minerals, phytochemicals and antioxidants (Hashemi, 2015). Barley is an ancient cereal grain, which, upon domestication, has evolved from a largely food's grain to feed and malting grain (Kumari and Kotecha, 2015). About 2% of the global barley production is used as food (Gupta et al., 2010). In Tunisia, barley is used as both feed (85%) and food (15%) (El Felahand Medimagh 2005). However, barley food use today remains important in some cultures around the world, particularly in Tunisia, a second centre of diversity for barley (Abdellaoui et al., 2010). Barley based foods represent the bulk of all foods consumed and their contribution to human nutrition and health should be taken seriously. Barley is nutritionally rich because it has a high carbohydrate concentration, moderate protein concentration, high dietary fiber content especially β-glucan and it is a good source of phosphorus and potassium (Kumari and Kotecha, 2015). Barley soup, barley bread, talbina, bssissa and "malthouth" are the first endemic barley-based food processed from the barley grain in Tunisia.Barley variety "Rihane" (BR) is the most used by the food industry for the production of these foods. In addition, many experimental studies have suggested an important role for BR in the prevention of colon cancer and cardiovascular disease (Lahouar et al., 2011; Lahouar et al., 2012; Lahouar et al. 2014<sub>a</sub>). Barley variety Rihane (BR) (Hordeumvulgare L.) a six-rowed improved cultivar, was registered in Tunisia in 1987, and obtained through a cross between a local landrace Atlas 46 (As46), improved material Arrivat (Avt) and Athenaïs (Aths). It's now a widely grown variety (more than 40% of total barley cultivated areas in Tunisia) (El FelahandMedimagh, 2005). This variety has contributed significantly to the increase of the barley national production in Tunisia but also it is cultivated in Morocco, Algeria, Libya, Lebanon, Egypt, Iraq, Iran, Afghanistan, Cyprus and China (Medimagh et al., 2012). Experimental studies have shown that the diet of BR has an important

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hypocholesterolemic effect. It could drastically decrease the levels of total cholesterol and low density lipoprotein (LDL) cholesterol in serum, but increases the levels of high density lipoprotein (HDL). Thus, long-term intake of BR has beneficial effects on lipid metabolism. The use of the azoxymethane (AOM) as colon specific carcinogen substance altered the lipid metabolism whereas the presence of BR could be a protective effect on lipid metabolism in the presence of a toxic substance such as AOM. In addition, this variety reduced the number of the incidence of aberrant crypt foci (precancerous stage) in rats azoxymethane-treated and therefore it has reduced colon cancer risk. (Lahouar et al. 2011; Lahouar et al. 2014). Lahouar et al. 2012 have showed that BR can have long-term beneficial effects on the composition of colonic microbiota. The BR diet increased the biodiversity and number of total bacteria after 12 weeks. BR increased also biodiversity of Bifidobacteria. Further, BR inhibited the growth of pathogenic bacteria such as E. coli. Consequently, this variety could be considered a prebiotic food. Lahouar et al. (2014) showed also that the BR diet significantly reduced the number of aberrant crypt per focus and altered their distribution. In addition, BR diet increased significantly the mucus secretion compared to control diet. The use of the azoxymethane as colon specific carcinogen substance altered the liver and lung architectures, whereas the presence of BR could be a protective factor for these organs.BR should be considered as an ideal healthy food. According to the characteristics of their ears, the Tunisian barley varieties (TBV) are ranked as barley with six row (Manel) and barley with two row(Roho and Tej). Even though barley is widely known thanks to its nutritional potential, in Tunisia, there is no published data about the chemical composition of barley varieties such as BR. The objective of this study was to evaluate the physico-chemical properties and nutritional characterizations of barley variety Rihane compared to the other Tunisian barleyvarieties (TBV) (Manel, Roho and Tej).

# Materials and Methods Barley samples

The "Rihane" variety, a six-rowed barley, was provided by the Field Crop Laboratory of INRAT. This variety was grown in 1.5/50-meter head-rows plots at the Agricultural Experimental Station of Béja, 100 km North-West of Tunisia. Three barley cultivars namely, Manel (six rows variety), Roho, Tej (two rows varieties) were procured from the Field Crop Laboratory of INRAT-Tunisia. The grain of each variety was cleaned and stored for evaluation. All tests were performed in triplicates on a dry weight basis.

# Morphological characteristics and technology of BR compared to TBV

The color and the form were the first recorded parameters. One thousand grain from each variety (BR and TBV) was weighed for thousand grain weight (TGW) determination. Specific weight (SW) of barley was measured using a chondrometer.

## Chemical analysis

All analyses were done in triplicate and the results are reported on a dry matter (DM) basis, determined by drying samples overnight at 105°C. Ash was determined by weight lost upon heating at 550°C for 5 h. Crude protein content was analyzed by the conventional Kjeldahl (Nx6.25) method. The fat content was determined by extraction in diethylether and petroleum ether. The obtained fat fraction of 25 mg was dissolved in 1 ml of benzene in order to prepare fatty acid methylesters. The glucids content was determined by subtraction. Total sugar content in extracts of BR and TBV was determined by the phenol–sulfuric acid method (Dubois 1956).

## Fatty acid composition profile

Fatty acid methylesters were analyzed on a Hewlett-Packard model 5890 series II gas chromatograph equipped with a flame ionization detector and a polar capillary column: HP Innowax cross-linked PEG, Carbowax 20 M (0.32 mm internal diameter, 30 m length and 0.25  $\mu$ m film thickness). The operational conditions were: injector temperature 220 °C; detector temperature 275 °C; column temperature 50 °C for 5 min then a gradient of 10 °C/min to 240 °C; carrier gas was nitrogen at a flow of 1.47 ml/min. Three injections were done.

# Total, insoluble and soluble dietary fibercontents

The determination of total dietary fiber (TDF) and insoluble dietary fiber (IDF) contents were carried out according the enzymatic-gravimetric method of AOAC method 991.43 described by Campos-Vega et al. (2010). At least, three determinations of each treatment were conducted. The soluble dietary fiber (SDF) was calculated by subtracting the IDF proportion from TDF.

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## **β-glucan content determination**

 $\beta$ -glucan was analysed using an McCleary method, which has been approved by the AAC (method 32-23) and the AOAC (method 995.16) (McClearyandCodd,1991).

#### Mineral contents

The determination of K and Na were made by flame photometry using a flame spectrophotometer (Turner, model 510). Similarly, the contents of Ca, Mg and Fe were determined by colorimetric method (Randox Antrim, Royaume-Uni). Phosphorus was determined spectrophotometrically using Scheel's method (Kamoun2008). Statistical analyses

All analyses were carried out in triplicate and the data were reported as means  $\pm$  standard error. P values<0.05 were regarded as significant. The statistical analysis was done by the STATVIEW 4.5. Analysis of variance (ANOVA) was used to assess the significance of differences among diverse varieties.

#### Results

## Morphological characteristics and technology of BR compared to TBV

Barley Rihane and Manelhavethe same color and the same form compared to two-row varieties (Table 1). TGW wasn't varied between types of varieties. It is higher in BR compared to the other varieties. There are no significant differences between specific weights of different varieties.

**Table 1**: Morphological characteristics and technology of BR compared to TBV.

	Rihane	Manel	Roho	Теј
Type	Six-row	Six-row	Two-row	Two-row
Form	slightly pyramidal	slightly pyramidal	elongated	elongated
Color	grayish-yellow	Greenish-yellow	yellowish-white	yellowish-white
$TGW^{c}$	$40.5{\pm}0.8^a$	$39.9 \pm 1.5^{a}$	$40\pm0.17^{a}$	$33.2 \pm 0.6^{b}$
$SW^{d}$	$65.15\pm1.24^{a}$	$66.5\pm2.02^{a}$	64.66±0.33 <sup>a</sup>	$63.33 \pm 0.66^a$

Values are the means of triplicate assays  $\pm$  Standard Error. Means in the same line with different lowercase letters are significantly different (p<0.0001).

## Chemical composition of BR compared to TBV

The chemical composition of BR and TBV is summarized in Figure 1. Fat content is significantly higher in BR (3.82 % DM) compared to two-row varieties (Roho and Tej). Crude protein is significantly higher in BR (11.37 % DM) compared to Roho but this content is significantly lower in BR compared to Manel and Tej (respectively 14.34 and 13.50% DM). BR is rich in glucids compared to Tej. These differences in terms of chemical composition between the 2- and 6-row barley are significant (P<0.0001). The total sugar content is lower in BR compared to two-row varieties.

<sup>&</sup>lt;sup>c</sup> Thousand grain weight (TGW) (g)

<sup>&</sup>lt;sup>d</sup> Specific Weight (SW) (kg/hl)

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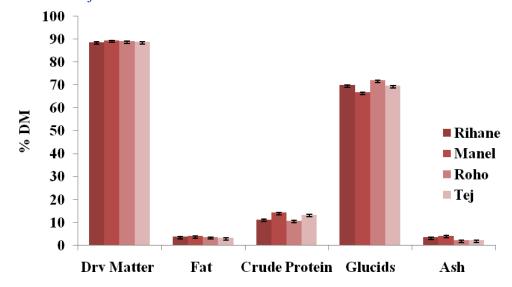


Figure 1: Chemical composition of barley Rihane (BR) compared to Tunisian barley varieties (TBV) (% DM).

# TDF, IDF and SDF contents of BR compared to TBV

Figure 2 shows the TDF, IDF, SDF contents and the ratio between IDF and SDF from BR and TBV. IDF content is higher in BR than in the other varieties. While the SDF and TDF contents are higher in BR variety compared to Manel. But these contents are lower compared to two-row varieties. The ratio of IDF and SDF is higher in BR compared to two-row varieties. TDF contents of BR and TBV (% DM) are found in the ranges from 31.47 to 41.70.

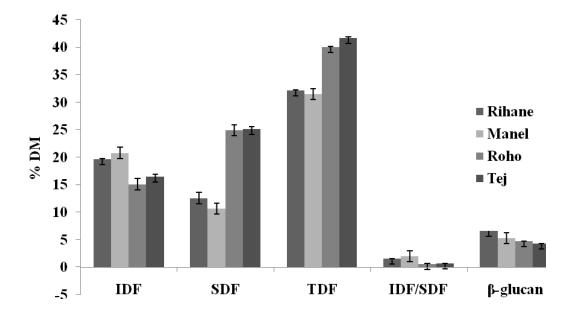


Figure 2: Insoluble, soluble and total dietary fiber contents of barley Rihane (BR) to Tunisian barley varieties (TBV) (% DM).

IDF=Insoluble Dietary Fiber;

SDF=Soluble Dietary Fiber;

TDF=Total Dietary Fiber.

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## β-glucan content of BR compared to TBV

The  $\beta$ -glucan content is significantly greater in barley Rihane than in other varieties, ranging from 4. 29 % in Tej to 6.64 % in Rihane. The  $\beta$ -glucan content is significantly greater in the barley six-row cultivars than in the barley two-row cultivars(Fig. 2).

## Fatty acid composition of BR compared to TBV

The results of fatty acid analysis for BR variety and TBV are listed in Table 2. The  $\alpha$ -Linolenic acidis the major component for BR and TBV. This content is higher in BR (29.03 % Total Fatty Acids) compared to the other varieties. Oleic acid is higher in Rihane and Manel than others varieties. Palmitoleic acid content is lower in BR variety than in other varieties (Roho and Tej). The  $\alpha$ -Linolenic acid is the major component for BR and TBV. Oleic acid was higher in Rihane than other varieties.

# Mineral contents of BR compared to TBV

The mineral (ash) content of BR and TBV varied from 2.34 to 4.25 (Table 3). 2.0 to 3.0%, depending on genotype. This content is higher in BR than in the two-row varieties. The analysis of major minerals (iron, magnesium, calcium, sodium, potassium and phosphorus) of BR and TBV showed that phosphorus and potassium are the major minerals. These minerals are highest levels in Tunisian barley varieties compared to the other minerals.BR is relatively rich source of phosphorous and potassium. However it can be seen that BR grains contain moderate amounts of sodium, calcium and magnesium (78, 70.29 and 49.57mg/100g DM respectively). But this variety is poor source of iron (Table 3). The mineral content is higher in BR than in other varieties.

**Table 2**: Fatty acid profile of BR compared to TBV (%).

Fatty acid composition	Rihane	Manel	Roho	Tej
C12:0	ND	ND	ND	ND
C14:0	$1.23\pm0.04^{a}$	$1.71\pm0.01^{b}$	$0.98\pm0.01^{c}$	$0.43 \pm 0.07^d$
C16:0	$9.61\pm0.07^{a}$	$8.20\pm0.01^{b}$	$7.79\pm0.05^{\circ}$	$6.71 \pm 0.03^{d}$
C17:0	$0.51 \pm 0.05^{a}$	$2.00\pm0.02^{b}$	$0.26\pm0.01^{c}$	$0.66 \pm 0.02^d$
C18:0	$2.44{\pm}0.06^{a}$	$3.94\pm0.03^{b}$	4.42±0.08°	$5.55 \pm 0.08^d$
C20:0	$2.56\pm0.03^{a}$	$0.95 \pm 0.02^{b}$	4.60±0.06°	$3.54 \pm 0.02^d$
C22:0	1.20±0.01a	$0.49 \pm 0.06^{b}$	$0.14\pm0.02^{c}$	$0.73 \pm 0.08^d$
C24:0	$0.96\pm0.01^{a}$	$0.40\pm0.04^{b}$	0.21±0.01°	$0.74 \pm 0.04^d$
SFA	$18.50\pm0.25^{a}$	17.70±0.19 <sup>b</sup>	18.41±0.23a	18.37±0.35ac
C14:1	$0.94{\pm}0.02^{a}$	$2.04 \pm 0.03^{b}$	$0.22\pm0.02^{c}$	$0.81 {\pm} 0.08^a$
C16:1 w7	$5.35{\pm}0.03^{a}$	$3.28{\pm}0.03^{b}$	12.74±0.09°	$11.45 \pm 0.05^{d}$
C18:1 w9	$16.15\pm0.03^{a}$	$15.95 \pm 0.01^{b}$	12.61±0.09°	$10.32 \pm 0.05^d$
C18:1 w7	$0.68{\pm}0.01^{a}$	$1.26 \pm 0.02^{b}$	2.91±0.03°	$3.47 \pm 0.09^d$
C20:1 w9	$3.19\pm0.07^{a}$	$0.53 \pm 0.02^{b}$	$2.30\pm0.02^{c}$	$2.90\pm0.04^{d}$
C22:1	$1.10\pm0.05^{a}$	5.60±0.03b	$1.07 \pm 0.02^a$	1.72±0.04°
C24:1	$1.33\pm0.01^{a}$	$0.78 \pm 0.06^{b}$	$0.50\pm0.03^{c}$	$0.23 \pm 0.05^d$
MUFA	$28.72\pm0.22^{a}$	29.45±0.21b	32.35±0.29°	30.91±0.41 <sup>d</sup>
C18:2 w6	$13.26 \pm 0.03^a$	$13.24 \pm 0.02^a$	$12.31 \pm 0.04^{b}$	14.52±0.04°
C18:3 w3	$29.03 \pm 0.02^a$	$23.29{\pm}0.02^{b}$	$20.64 \pm 0.05^{c}$	19.04±0.03 <sup>d</sup>

# doi:10.21010/ajtcam.v14i1.33

C20:4 w6	1.03±0.01 <sup>a</sup>	$1.62\pm0.06^{b}$	$3.27\pm0.04^{c}$	$2.23\pm0.04^{d}$
C20:5 w3 (EPA)	$4.03\pm0.03^{a}$	5.53±0.09 <sup>b</sup>	$1.59\pm0.05^{c}$	$2.11\pm0.01^{d}$
C22:4 w6	$0.65\pm0.01^{a}$	$4.38\pm0.03^{b}$	$0.40\pm0.01^{c}$	$1.33 \pm 0.05^{d}$
C22:5 w3	$1.07\pm0.06^{a}$	$1.59\pm0.06^{b}$	8.93±0.03°	$9.56\pm0.09^{d}$
C22:6 w3 (DHA)	$3.70\pm0.06^{a}$	$3.20\pm0.03^{b}$	$2.09\pm0.05^{c}$	$1.93\pm0.04^{d}$
PUFA	52.78±0.23a	$52.85 \pm 0.30^{b}$	49.24±0.27a	50.73±0.29ac
SFA/PUFA	$0.35^{a}$	0.33 <sup>b</sup>	$0.37^{c}$	$0.36^{d}$
U/S <sup>e</sup>	2.62 <sup>a</sup>	2.34 <sup>b</sup>	2.85°	$2.86^{d}$

Values are means of triplicate assays  $\pm$  Standard Error.Means in the same line with different lowercase letters are significantly different (P<0.0001).

ND: Not detected.

**Table 3**: Mineral contents of BR compared to TBV (mg/100g DM).

Minerals	Rihane	Manel	Roho	Tej
Fe	$0.098\pm0.06^{a}$	0.176±0.12b	$0.168\pm0.10^{b}$	0.257±0.15°
Mg	$49.572\pm0.86^{a}$	$55.89 \pm 1.08^{b}$	$52.00\pm0.33^{c}$	$71.19\pm0.37^{d}$
Ca	$70.29\pm0.07^{a}$	$69.60\pm0.13^{b}$	$68.30\pm0.28^{c}$	$62.80\pm0.05^{d}$
Na	$78.00\pm0.11^{a}$	$78.00\pm0.19^{a}$	$125.00\pm0.15^{b}$	$194.00\pm0.20^{c}$
K	$480.00\pm3.6^{a}$	$470.00\pm0.6^{b}$	$461.00\pm0.34^{c}$	$313.00\pm0.55^{d}$
P	$570.00\pm2.88^a$	$520.00\pm0.42^{b}$	$440.00\pm6.08^{c}$	$490.00\pm1.15^{d}$

Values are means of triplicate assays  $\pm$  Standard Error. Means in the same row followed by different superscript letters are significantly different (P<0.0001).

## Discussion

The importance of functional foods, nutraceuticals and other natural health products has been well recognized in connection with health promotion and risk reduction of chronic diseasesEl (Sohaimy, 2012). Whole foods such as whole grains often serve as a concentrated source of components with health beneficial effects. In recent years, the research has been oriented to cereal especially the barley because of its potential to enable the development of functional foods(Adil et al., 2012). Several of the nutrients in barley have known potential, if in adequate amounts, for reducing risk factors for civilization diseases (cardiovascular disease, type-2 diabetes, some cancers...) (Gubatz and Shewry 2011). This study showed the physico-chemical properties and nutritional characterization of bioactive components barley variety Rihane which have cholesterol lowering effect and preventif factor of colon cancer. In this present study, the number of sample is reduced as it was difficult to have untreated varieties. The environmental factors and genotype x environment interactions do not have significant effects on barley grain composition. In additional, the comparisons were made on samples grown under the same conditions. TGW is not varied between the different varieties. The results are consistent with those of Öztürk et al. (2007) which showed that TGW was the most important yield component. There are no significant differences between specific weights of different varieties. Specific weight is physical factor which may influence indirectly the energetic value due to the negative correlation between specific weight of the grain and its concentration of dietary fibers (Wilkinson et al. 2003).

The results obtained on glucids contents are in agreement with those previously reported for Brazilian varieties (Vieira and Alicia 2004). These compounds representing a major source of energy for humans (Mahdi et al. 2008). Protein concentration in Canadian barley genotypes varied from 10 to 15% however the range is wider (7 to 25%) in other improved genotypes depending on end use (Newman and Newman 2005). Barley with high protein concentration (> 15%) is not used for malting as it requires a long steeping time, it has erratic germination and produces low malt extract (Swanston and Molina-Cano 2001). The concentration of lipids in barley endosperm (mostly in the embryo) ranged from 2.0 to 7.3% depending of extraction methods and genotype (Qian et al. 2009). The total sugar content is lower in BR compared to other varieties. Holtekjølen et al. (2008) showed that barley contained simple sugars such as glucose, fructose and maltose with concentrations ranging from 0.1 to 0.2% DM.

The results obtained on dietary fibers contents are in accordance with those found by Anderson et al. (1999) which showed that TDF content of American barley variety (Prowashonupan) was 34% DM. Ragaee et al. (2006) showed that whole grains of barley and rye contained the highest level and it could be considered as a good source of

e Unsaturation ratio = (16:1 + 18:1 + 18:2)/(12:0 + 14:0 + 16:0 + 18:0).

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soluble dietary fiber. Insoluble dietary fiber content markedly varied among whole grains of cereal and flours ranging from 13.5 % to 22.1% in whole grains and from 1.9 % to 3.0 % in wheat flours. Total dietary fiber ranged from 11 to 34 % DM and soluble dietary fiber from 3 to 20 % DM (Mahdi et al. 2008). SDF and IDF are known to play different physiological roles in human health. The IDF/SDF proportion decreases with the row type and in this way it increases the physiological quality of the fiber.

Some studies showed that barley had high content of soluble dietary fiber mainly  $\beta$ -glucan (Mahdi et al. 2008). Given as several studies showed that soluble dietary fiber of barley ( $\beta$ -glucan) have health benefits for humans.  $\beta$ -glucan delays gastric emptying, lowers serum cholesterol and attenuates the postprandial glycemic response.  $\beta$ -glucan has also been reported to possess anti-cancer properties. The physiological functions of barley  $\beta$ -glucans also seem to be associated with fermentation of  $\beta$ -glucan in the colon, consequence in production of short-chain fatty acids, which prevents cholesterol biosynthesis (Brownlee 2011). Therefore, biologically active constituents of barley, that promote physiologically beneficial effects, are dietary fiber especially  $\beta$ -glucan. Barley Rihane has been associated with the lowering of serum LDL-cholesterol, a risk factor for heart disease. LDL-cholesterol is involved in the development of atherosclerosis. Thus, the soluble fiber component of barley Rihane grains ( $\beta$ -glucan) could be the responsible for the hypocholesterolemic effects. The present study showed also that the factors which have an important protective effect of against colon cancer or adenoma, could be  $\beta$ -glucan.

In another study, Lahouar et al. (2014b) revealed that BR contains reasonable levels of total phenolic content and antioxidant properties. These authors showed that BR has important nutritional values due to its particularly high content of bioactive phenolic compounds, such as total flavonoid and condensed tannin contents. Thus, whole grains of BR are good sources of dietary fiber mainly  $\beta$ -glucan and other bioactive compounds, which could work synergistically to optimize human health. These components are thought to protect the body from damaging free radicals and may have a role to play in disease prevention.

The results of fatty acid analysis are similar to those reported by GeiXler et al. (2003) who demonstrated that the main components of the unsaturated fatty acids in barley were oleic acid, linoleic acid and  $\alpha$ -linolenic acid. Lipid content and fatty acid composition of barley vary with the date of harvest as well as genotype and environmental and weather conditions before and after flowering. A relationship has also been found between barley fatty acid composition and kernel size as well as a similarity between the lipid composition of small mature grain and immature grain (deMan and Bruyneel, 1987). This study showed that BR might serve as a good source of polyunsaturated fatty acid. Compared to carbohydrates and protein, content of lipids in most BR is relatively low. Their contribution toward the nutritional value as well as storage stability of barley-based food or feed, however, is important.

Minerals which affect the nutritional value of the barley are divided into macro- and micro-elements based on concentration in foods. The macro-elements include calcium, phosphorus, potassium, magnesium and sodium. The rest are iron, manganese, zinc, selenium and cobalt which are the nutritionally important micro-elements in the barley kernel (Marconi et al. 2000). The results obtained for different minerals (magnesium, calcium, sodium, potassium and phosphorus) are higher in BR compared to others varieties. The major mineral compounds in BR are phosphorus and potassium. It would appear that BR grains could provide reasonable amounts of most minerals needed for adequate nutrition.

## Conclusion

Barley Rihane has significant nutritional characterizations compared to others Tunisian barleys varieties. BR grain is good source of many nutrients including dietary fiber especially  $\beta$ -glucan, minerals (potassium and phosphorus), and unsaturated fatty acids. It is possible to conclude that BR is a convenient source for the production of high fiber functional foods. Its consumption can considerably contribute to the improvement of the health state of the population.

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